

## **IN THE CLAIMS**

1-19. (canceled)

20. (new) A polypeptide comprising the S1 subunit of pertussis toxin wherein the S1 subunit is modified by the substitution in one or more sites selected from the group consisting of tyrosine in position 8, arginine in position 9, phenylalanine in position 50, threonine in position 53, glutamic acid in position 129, glycine in position 121, alanine in position 124, aspartic acid in position 109, glycine in position 99, arginine in position 135, threonine in position 159 and tyrosine in position 111 with another amino acid capable of destroying or reducing the toxicity of the S1 subunit.

21. (new) The polypeptide of claim 20 wherein at least glutamic acid in position 129 of the S1 subunit is substituted with another amino acid.

22. (new) The polypeptide of claim 21 wherein said polypeptide is further modified by the substitution in one or more sites selected from the group consisting of tyrosine in position 8, arginine in position 9, phenylalanine in position 50, threonine in position 53, glycine in position 121, alanine in position 124, aspartic acid in position 109, glycine in position 99, arginine in position 135, threonine in position 159, and tyrosine in position 111 with another amino acid capable of destroying or reducing the toxicity of the S1 subunit.

23. (new) The polypeptide of claim 22 wherein arginine in position 9 of the S1 subunit is substituted with another amino acid.

24. (new) The polypeptide of claim 20 wherein said polypeptide further comprises at least one of the S2, S3, S4, and S5 subunits of the pertussis toxin.

25. (new) The polypeptide of claim 24 wherein said polypeptide comprises the S2,

S3, S4, and S5 subunits and said S2, S3, S4, and S5 subunits have the same arrangement as that present in natural pertussis toxin.

26. (new) The polypeptide of claim 20 wherein tyrosine in position 8 of the S1 subunit is substituted with aspartic acid and wherein arginine in position 9 of the S1 subunit is substituted with glycine.

27. (new) The polypeptide of claim 20 wherein phenylalanine in position 50 of the S1 subunit is substituted with glutamic acid and wherein threonine in position 53 of the S1 subunit is substituted with glycine.

28. (new) The polypeptide of claim 20 wherein glycine in position 99 of the S1 subunit is substituted with glutamic acid.

29. (new) The polypeptide of claim 20 wherein the glycine in position 121 of the S1 subunit is substituted with glutamic acid.

30. (new) The polypeptide of claim 20 wherein the alanine in position 124 of the S1 subunit is substituted with aspartic acid.

31. (new) A method for the preparation of a polypeptide of claim 20, comprising:

- a) modifying by direct mutagenesis a DNA molecule coding for an S1 subunit of pertussis toxin a base sequence of which codes for an amino acid selected from the group consisting of (1) tyrosine in position 8, (2) arginine in position 9, (3) phenylalanine in position 50, (4) threonine in position 53, (5) glutamic acid in position 129, (6) glycine in position 121, (7) alanine in position 124, (8) aspartic acid in position 109, (9) glycine in position 99, (10) arginine in position 135, (11) threonine in position 159, and (12) tyrosine in position 111 of the S1 subunit with a base sequence that codes for an amino acid of interest;

b) constructing a hybrid plasmid linking a cloning vector with the DNA molecule;

c) transforming a host microorganism with the hybrid plasmid;

d) cultivating the transformed host microorganism in a suitable culture medium;  
and

e) recovering the polypeptide from the culture medium or from the host microorganism.

32. (new) The method of claim 31 wherein the DNA molecule is the gene coding for the S1 subunit is contained in the pertussis toxin operon.

33. (new) The method of claim 32 wherein the DNA molecule further encodes at least one of the S2, S3, S4, or S5 subunits of the pertussis toxin.

34. (new) The method of claim 33 wherein the DNA molecule is the operon that codes for the pertussis toxin.

35. (new) The method of claim 32 wherein the host microorganism is selected from the group consisting of *E. coli*, a bacillus, and a yeast.

36. (new) The method of claim 35 wherein the microorganism is *E. coli*.

37. (new) An antipertussis vaccine comprising a therapeutically effective quantity of at least one polypeptide of claim 21.

38. (new) An antipertussis vaccine comprising a therapeutically effective quantity of at least one polypeptide of claim 22.

39. (new) An isolated DNA molecule comprising a nucleotide sequence coding for a polypeptide comprising the S1 subunit of pertussis toxin wherein bases coding for one or more

sites of the S1 subunit selected from the group consisting of (1) tyrosine in position 8, (2) arginine in position 9, (3) phenylalanine in position 50, (4) threonine in position 53, (5) glutamic acid in position 129, (6) glycine in position 121, (7) alanine in position 124, (8) aspartic acid in position 109, (9) glycine in position 99, (10) arginine in position 135, (11) threonine in position 159, and (12) tyrosine in position 111 are substituted with bases coding for another amino acid capable of destroying or reducing toxicity of the S1 subunit.

40. (new) The DNA molecule of claim 39 wherein at least the bases of the DNA molecule coding for glutamic acid in position 129 of said S1 subunit are substituted with bases coding for another amino acid.

41. (new) The DNA molecule of claim 40 which is further modified by the substitution of bases coding for one or more amino acids of the S1 subunit selected from the group consisting of (1) tyrosine in position 8, (2) arginine in position 9, (3) phenylalanine in position 50, (4) threonine in position 53, (5) glycine in position 121, (6) alanine in position 124, (7) aspartic acid in position 109, (10) glycine in position 99, (11) arginine in position 135, (12) threonine in position 159, and (13) tyrosine in position 111 are substituted with bases coding for another amino acid capable of destroying or reducing toxicity of the S1 subunit.

42. (new) The DNA molecule of claim 41 wherein bases coding for arginine in position 9 of the S1 subunit are substituted with bases coding for another amino acid.

43. (new) The DNA molecule of claim 41 wherein the polypeptide contains at least one of the S2, S3, S4, or S5 subunits of the pertussis toxin.

44. (new) The DNA molecule of claim 43 wherein the polypeptide comprises the S2, S3, S4, and S5 subunits in the same arrangement as that present in natural pertussis toxin.

45. (new) The DNA molecule of claim 39 wherein bases coding for tyrosine in position 9 are substituted with bases coding for aspartic acid and wherein bases coded for arginine in position 9 of the S1 subunit are substituted with bases coding for glycine.

46. (new) The DNA molecule of claim 39 wherein bases coding for phenylalanine in position 50 are substituted with bases coding for glutamic acid and wherein bases coding for threonine in position 53 of the S1 subunit are substituted with bases coding for isoleucine.

47. (new) The DNA molecule of claim 39 wherein bases coding for glycine in position 99 of the S1 subunit are substituted with bases coding for glutamic acid.

48. (new) The DNA molecule of claim 39 wherein bases coding for glycine in position 121 of the S1 subunit are substituted with bases coding for glutamic acid.

49. (new) The DNA molecule of claim 39 wherein bases coding for alanine in position 124 of the S1 subunit are substituted with bases coding for aspartic acid.

50. (new) A method for immunizing a human against pertussis comprising administering an effective amount of a vaccine selected from the group consisting of the vaccine of claim 37 and the vaccine of claim 38.

51. (new) A method of preparing an antipertussis vaccine comprising formulating a therapeutically effective amount of a polypeptide in vaccine form, wherein the polypeptide is selected from the group consisting of the polypeptides of claims 21-25.

52. (new) An isolated preparation of *E. coli* selected from the group consisting of PTE 255-22 deposited as ATCC Accession No. 67542, PTE 255-28 deposited as ATCC Accession No. 67543, and PTE 255-41 deposited as ATCC Accession No. 67544.

Respectfully submitted,

BANNER & WITCOFF, LTD.

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By:

A handwritten signature in cursive script, appearing to read "Lisa M. Hemmendinger".

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